

SEQ ID NO:20 CCCAGGTGCA CACCAATGTG GTGGAT,
SEQ ID NO:21 GGACTGTGCG CGTTGTATAC CCTGC,
SEQ ID NO:22 ACTCCCGAAG CGAATGGCAC GTGGA,
SEQ ID NO:23 CATAGCTTGT GCCCGTGTGG CACGT,
SEQ ID NO:24 CCAAGACGAG ACCGTCAGAG CTGGT,
SEQ ID NO:25 AAGCTGTTGC CGCCATCAAA TAAACG, [or] and
SEQ ID NO:26 CTGCGTTAGA CCGAGAACTG TGGATAAAGG.

Please add the following new claims 39-42:

39. An aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

(a) first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

(b) third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m 's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μ molar, and

said first and second primers being selected from the group of primer sets selected from the group consisting of:

Primer set 1:

(a) SEQ ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

(b) SEQ ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

(a) SEQ ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

(b) SEQ ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3',

and

(c) a thermostable DNA polymerase present at at least 10 units/100 μ l.

40. A diagnostic test kit for the amplification of human cytomegaloviral DNA and a second target DNA comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m 's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in

the same amount within the range of from about 0.1 to about 2 μ molar, and

said first and second primers being selected from the group of primer sets selected from the group consisting of:

Primer set 1:

(a) SEO ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

(b) SEO ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

(a) SEO ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

(b) SEO ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3',

and

a thermostable DNA polymerase present at at least 10 units/100 μ l,

b) at least one additional PCR reagent, and

c) a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of a strand of hCMV DNA, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and being hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

wherein said first capture probe is selected from the group consisting of:

SEO ID NO:5 5'-GGTGTACCCC CCAGAGTCCC CTGTACCCGC-3',

SEO ID NO:6 5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',

SEO ID NO:7 5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3',

and

SEO ID NO:8 5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3'.

41. A diagnostic test kit for the amplification of human cytomegaloviral DNA and a second target DNA comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

each of said first and second primers being present in the same amount within the range of from about 0.1 to about 2 μ molar and having a T_m within the range of from about 65 to about 74°C, said primer T_m 's being within about 5°C of each other, [and]

said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

said first and second primers being selected from the group of primer sets selected from the group consisting of:

Primer set 1:

(a) SEO ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

(b) SEO ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

(a) SEO ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

(b) SEO ID NO:4 5'-CACCACGCAG CGGCCCTGA TGTTT-3',

b) a separate aqueous composition buffered to a pH of from about 7 to about 9, and comprising third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA and which are separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides,

each of said third and fourth primers being present in the same amount of from about 0.1 to about 2 μ molar and having a T_m within the range of from about 65 to about 74°C, said third and fourth primer T_m 's being within about 5°C of each other and within about 5°C of the T_m 's of said first and second primers, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides,

c) included in either a) or b), a thermostable DNA polymerase present at at least 10 units/100 μ l,

d) at least one additional PCR reagent, and

e) a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of a strand of hCMV DNA, said first capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m 's which differ by no more than about 15°C, and

wherein said first capture probe is selected from the group consisting of:

SEQ ID NO:5 5'-GGTGTACCCC CCAGAGTCCC CTGTACCCGC-3',

SEQ ID NO:6 5'-GACACAGTGT CCTCCGCTC CTCCTGAGCA-3',

SEQ ID NO:7 5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3',

and

SEQ ID NO:8 5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3'.

42. A method for the amplification and detection of human cytomegaloviral DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of hCMV DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m 's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μ molar, and

said first and second primers being selected from the group of primer sets selected from the group consisting of:

Primer set 1:

(a) SEO ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

(b) SEO ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3'.

and

Primer set 2:

(a) SEO ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

(b) SEO ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3',a

and

ii) the following additional PCR reagents: a thermostable DNA polymerase present in an amount of at least 10 units/100 μ l, a DNA polymerase cofactor and at least one dNTP, any or all of said additional PCR reagents being in the same or a different composition as defined in i),

to simultaneously amplify said opposing hCMV DNA strands and the opposing second target DNA strands wherein, in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from about 62 to about 75°C and carried out within 120 seconds,

B) capturing one of said amplified hCMV DNA strands with a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of said hCMV DNA strand, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

capturing one of said amplified second target DNA strands with a second capture reagent comprising a second capture probe specific to a nucleic acid sequence of said second target DNA strand, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m 's which differ by no more than about 15°C, and

wherein said first capture probe is selected from the group consisting of:

SEO ID NO:5 5'-GGTGTCACCC CCAGAGTCCC CTGTACCCGC-3',

SEO ID NO:6 5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',

SEO ID NO:7 5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3',

and

SEO ID NO:8 5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3',

and

C) simultaneously detecting said captured amplified hCMV DNA strand and said captured amplified second target DNA strands as a simultaneous determination of the presence of hCMV DNA and said second target DNA.

REMARKS

The specification has been amended on page 33 to correct a typographical error. The Examiner's thorough review is appreciated.

As requested by the Examiner, each Figure has been amended to identify units on the y-axis and to provide descriptive indications on the x-axis. A separate letter to the Official Draftsman is enclosed with the corrected drawings in proper form. Approval of the corrected drawings is requested.

Claim 38 has been amended a second time as is discussed in more detail below in response to the Examiner's rejection.

New Claims 39-42 are submitted as more narrow embodiments of original Claims 1, 12, 19 and 22. They incorporate the features of canceled Claims 9, 15, 32 and 34 into the independent claims. Applicants respectfully request entry of these amendments in their sincere attempt to expedite prosecution. No new issue is raised and no new matter is presented since the incorporated language is taken from the canceled claims which have already been searched. It is also believed that these new claims are more readily distinguishable over the cited prior art.

Rejection Under 35 U.S.C. 112, Second Paragraph

Claims 1-8 and 10-38 have been rejected as being indefinite for failing to recite specific oligonucleotide sequences. As far as it applies to